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(54) Title: HYBRID PEPTIDES AND METHODS OF THEIR USE

(57) Abstract

A new class of hybrid peptides having domains which are analogues of receptor interaction sites on an adhesive protein have been developed. These hybrid peptides are useful in methods of inhibiting aggregation of platelets and adhesion of platelet and fibrin thrombi to vessel walls. Methods of producing synthetic substitutes for fibrinogen, von Willebrand factor and other adhesive proteins are also disclosed.

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HYBRID PEPTIDES AND METHODS OF THEIR USE

Reference to Government Rights

The present invention was made, in part, in the course of work under Research Grant HL-33014 from the National Institutes of Health, United States Public Health Service.

Background of the Invention

The present invention relates to synthetic peptide analogues of active sites on proteins and their uses. More particularly, the invention relates to hybrid linear peptides which have sites that mimic at least two interaction sites of proteins with receptor sites, e.g., receptor sites for fibrinogen on platelets.

The interaction between platelets and plasma proteins such as fibrinogen has been extensively studied. Separate platelet receptor recognition domains or interaction sites have been located on the gamma chain and alpha chain of fibrinogen. See Hawiger, J., Timmons, S., Kloczewiak, M., Strong, D. D., and Doolittle, R. F., Proc. Natl. Acad. Sci. U.S.A. 79, p. 2068 (1982). The platelet receptor recognition domain on the gamma chain is located between residues 400-411 (γ400-411), and the alpha chain platelet receptor recognition domain is thought to be related to two loci (α95-97 and α572-575). The sequences on the gamma chain (HHLGGAKQAGDV) are

not homologous to the common areas flanking th sequence RGD on the alpha chain.

The native human fibrinogen alpha chain fragment encompassing residues α518-584 and the gamma chain fragment encompassing residues γ385-411 can each independently inhibit the ADP- or thrombin-induced interaction between the glycoprotein fibrinogen receptor on platelets and fibrinogen. However, because of the disparity in structure between the alpha and gamma platelet receptor recognition domains, functional cross-specificity testing had not been carried out. As is demonstrated herein, synthetic peptide analogues of the alpha chain can inhibit gamma chain binding and visa-versa. The use of synthetic peptide analogues in platelet-binding systems, as well as their medical applications, is further discussed in United States Patents Nos. 4,661,471, 4,666,884, and 4,703,039, all based on applications of some of the present inventors.

Fibrinogen, fibrin, and von Willebrand factor are important in the formation of hemostatic platelet plugs and the initiation of thrombotic lesions. Blockage caused by these plugs and the damage caused by thrombotic lesions are major factors in heart disease and stroke. Much research has been directed towards developing drugs that will dissolve already formed blood clots but many of these drugs, e.g., see recent reports on the use of streptokinase and tissue plasminogen activator, have their drawbacks. While enzymatic methods of dissolving

clots may help minimize the aft r-eff cts of h art attacks, a pharmaceutical preparation which will inhibit platelet adhesion and clumping may prevent the initial blockage of blood vessels responsible for cardiac or cerebral infarction.

Alternatively, the manufacture and use of molecules which promote platelet aggregation, or adhesion to blood vessels may have a variety of uses. For example, a number of patients, e.g., some bleeders, may be missing fibrinogen or von Willebrand factor due to genetic deficiencies or due excess consumption in circulation. A synthetic platelet aggregation or adhesion promoting molecule can assist in platelet plug formation and attachment of plugs to blood vessels to arrest bleeding and help these patients to lead normal lives.

Accordingly, an object of the invention is to provide hybrid linear peptide analogues which mimic at least two distinct protein domains which interact with receptor sites on platelets.

Another object of the invention is to provide a method in inhibiting the interaction between fibrinogen, von Willebrand factor, and other adhesive proteins with platelets by the use of hybrid linear peptide analogues of the interaction domains of the adhesive proteins.

A further object of the invention is to provide synthetic aggregation or adhesion promoting molecules formed of multimers of hybrid linear

peptide analogues of at least two interaction domains on fibrinogen or von Willebrand factor.

These and other objects and features of the invention will be apparent from the following description and drawings.

Summary of the Invention

The present invention features hybrid linear peptides which have interaction sites that are analogous to interaction sites on a native protein. These interaction sites on the native protein will react with a receptor recognition site, e.g., a platelet receptor recognition site for the protein. The interaction sites are physically distinct in the native protein.

In particular, the invention features a hybrid linear peptide of the form X_1-X_2 where X_1 is an analogue of a first interaction site on a protein for a first receptor domain of that protein and X_2 is an analogue for a second interaction site on the protein for a second receptor domain. The receptor can be a glycoprotein or any other type of receptor which reacts with a linear protein fragment. The domains or interaction sites are distinct on the native form of the protein. The preferred receptor is the glycoprotein receptor site on human platelets for fibrinogen, fibrin, von Willebrand factor, or other adhesive proteins.

Fibrinogen is th preferred protein whose sit s form the basis of the analogues and X_1 and X_2 are preferably analogues of the interaction sites for platelets on the gamma and alpha chains, respectively. The X_1-X_2 preferred hybrid peptide has, therefore, at least a portion of the platelet receptor recognition domain from the gamma chain near its amino terminal end and a portion of the platelet recognition receptor domain from the alpha chain near the carboxyl terminal end. Alternatively, the arrangements of these domains can be reversed. The most preferred X_2 or carboxyl terminal portion for fibrinogen-platelet analogue systems is a tetrapeptide selected from a group consisting of RGDV, RGDN, RGDF, RGDY, RGDS, RGDM, and RGDC. The most preferred X_1 portion is a linear peptide fragment of 2-13 residues comprising an active portion of the sequence HHLGGAKQAGDV or substitute analogues thereof. The symbols designating the amino acids are those standard in peptide chemistry and are set forth in Table I.

TABLE I

Amino Acid Symbols

Amino Acid	One-letter Symbol
Alanine	A
Arginine	R
Asparagine	N
Aspartic Acid	D
Asm + Asp	B
Cysteine	C
Glutamine	Q
Glutamic Acid	E
Gln + Glu	Z
Glycine	G
Histidine	H
Isoleucine	I
Leucine	L
Lysine	K
Methionine	M
Phenylalanine	F
Proline	P
Serine	S
Threonine	T
Tryptophan	W
Tyrosine	Y
Valine	V

The invention also features methods for inhibiting the interaction of platelets with native adhesive proteins, e.g., fibrinogen, primarily as an antiaggregation mechanism. This method has the steps of forming the hybrid linear peptide of the invention, reacting the hybrid peptide with the platelets, and exposing the platelets to the adhesive protein. Pre-incubation or pre-reaction of the peptides with the platelets and exposure of the peptides to the adhesive protein allows the hybrid peptides to interact with the platelets, thereby filling the receptor site, and prohibiting the adhesive protein platelet interaction. This type of procedure can be used to inhibit the interaction of fibrinogen, fibrin, von Willebrand factor, and other adhesive protein with platelets, thereby inhibiting hemostatic plug formation and adhesive of platelets to vessel walls.

The invention further features a method of promoting aggregation and/or adhesion of platelets as part of a hemostatic plug formation process. This method is based on the use of multimers of the hybrid peptide of the invention to form synthetic aggregation or adhesion-promoting molecules. These multimers are either cross-linked plural copies of the hybrid or plural hybrids may be linked to a protein or other analogous backbone. In either case, the multimers have the ability to react with receptors on separate molecules, thereby cross-linking the molecules. The method of the invention starts by manufacturing these synthetic aggregation or adhesion promoting molecules and then

incubating th platelets with these mol cules under conditions which promote the interaction between the receptors and the synthetic molecules. Conditions which help promote this type of adhesive interaction include activation of platelets with ADP, thrombin epinephrine, or other agonists.

Brief Description of the Drawings

Figure 1 illustrates that both the fragment corresponding to the platelet receptor recognition site on the alpha chain (RGDS) and the fragment corresponding to the platelet receptor recognition site on the gamma chain (γ400-411) will inhibit the aggregation of platelets by gamma chain or alpha chain multimers;

Figure 2 shows the inhibition of binding of ^{125}I-Fibrinogen to human platelets by the incubation with gamma chain fragments (Fig. 2A) and alpha chain fragments (Fig. 2B-D); and

Figure 3 illustrates the inhibition of binding of ^{125}I-Fibrinogen by seven different hybrid peptides within the scope of the invention.

Detailed Description of the Invention

The present invention is based, in part, on the finding that platelet receptor recognition domains located on the gamma and alpha chains of human fibrinogen are functionally cross-inhibited by synthetic peptides derived from these chains despite

the lack of homolog us amino acid sequences. Figure 1 illustrates this phenomenon of cross-inhibition by synthetic peptide analogues of sequences present in the gamma and alpha chains. Figure 1A shows the inhibition of the interaction between platelets and gamma chain multimers, while Figure 1B shows the inhibition of the interaction of platelets and alpha chain multimers. More particularly, Figure 1A illustrates an experiment whereby platelets were pre-mixed with a buffer, a fragment corresponding to the alpha chain platelet receptor recognition domain of fibrinogen (RGDS), or the gamma chain platelet receptor recognition domain of fibrinogen (γ400-411). After ADP-treatment, 5 μM of gamma chain multimers were added to the treated platelets and the transmission percentage was measured. As is evident from the Figure, the buffer had no effect on the percent transmission while the γ400-411 fragment clearly inhibited the interaction between the gamma chain multimers and the platelets. This is expected since the γ400-411 fragment is the interaction site on the gamma chain. Surprisingly, the alpha chain fragment (RGDS) was equally effective in inhibiting the interaction with the gamma chain multimers. An additional parallel experiment, as shown in Figure 1B, illustrates that the alpha and gamma chain fragments will also inhibit the interaction between alpha chain multimers and ADP-treated platelets. Based on this finding, a "Checkerboard" analysis experiment was run testing a variety of concentrations of the alpha and gamma chain fragments to determine whether physical

mixtures interacted. Table II shows the results of this exp riment.

TABLE II

Checkerboard Analysis of the Inhibitory Potency of a Combination of Dodecapeptide γ400-411 and RGDS Toward ^{125}I-Fibrinogen Binding to Platelets.

γ400-411 \ RGDS	0	5 μM	10 μM	15 μM
0		30%	46%	58%
5 μM	2%	28%	54%	65%
15 μM	10%	55%	60%	72%
30 μM	40%	66%	65%	83%
60 μM	61%	73%	77%	93%

Concentrations of a dodecapeptide (γ400-411) ranging from 5-60 μM and concentrations of a tetrapeptide (RGDS) ranging from 5-15 μM were mixed as shown to test for inhibition of radiolabelled fibrinogen binding to platelets. Purified fibrinogen, iodinated with ^{125}I using standard iodine monochloride methods and having a specific radioactivity of 3×10^7 cpm/mg was used. Human platelets, separated from plasma proteins and suspended in HEPES balanced salt buffer, pH 7.35, were mixed with given concentrations of the peptide for five minutes. The labelled fibrinogen (33 μg/0.5 mL) was added, followed by 5 μM ADP. The binding experiments were carried out a room temperature, without stirring, in a final volume of 0.5 mL which contained 1×10^8 platelets. The results of Table II are shown as percent binding of a control value determined by a test without any peptide fragment added.

As is evident from Table II, the inhibition is purely additive at low concentrations of peptide while as high concentrations, there is a fall-off of the additive effect. In no instance is a synergistic effect shown.

The results of the foregoing experiments lead to further experimentation by the inventors on the structure of the binding domain on fibrinogen for human platelets. As part of this experimentation, hybrid peptides which form the basis of the present invention were manufactured and tested. As is evident from the following Example, surprising

results were obtained when relatively short hybrid peptides were formed having binding domains from both the alpha and gamma chain of fibrinogen in the same molecule.

EXAMPLE.

In this Example, a series of hybrid peptides having a variety of analogues of receptor domains from the alpha and gamma chains of fibrinogen were tested for inhibition of binding of ^{125}I-Fibrinogen to ADP-treated human platelets and the inhibitory effect of the peptides on the aggregation of the ADP-treated platelet-rich plasma. The results clearly showed that the hybrid peptides of the invention yield surprisingly good inhibition of the fibrinogen-platelet interactions.

The peptides were synthesized by solid phase methods either manually, see Kloczewiak et al., Biochemistry, 23, p. 1767 (1984), or with a Biosearch 9500 Peptide Synthesizer. The peptides were cleaved from the resin with HF and concentrations identified using standard procedures (see Kloczewiak et al., Ibid.). The inhibition of aggregation of platelet-rich plasma was measured photometrically in a Payton dual channel aggregometer (Payton Associates, Buffalo, New York). Aggregation was measured after the addition of ADP (5 μM) using a percentage of maximum transmission (Tmax) and rate (slope value) which represented a change in one minute along a tangent line to the steepest increase

in light transmission. Timmons et al., Trans. Assoc. Am. Phys., 99, p. 226 (1986).

Binding of ^{125}I-Fibrinogen was carried out as previously described, with a five minute incubation of the peptide and the plasma-free platelets in HEPES buffer followed by treatment with ADP and fibrinogen.

Although the peptides were synthesized using standard solid phase peptide synthesis procedures, recombinant methodology could be use to form the hybrid peptides within the scope of the present invention. Therefore, as used herein, the term "hybrid peptide" or "synthetic peptide" means and includes hybrid or synthetic peptides made using any procedure including classical peptide synthesis and recombinant techniques.

Figure 2 illustrates the inhibition of ^{125}I-Fibrinogen binding by two peptide fragments, γ400-411 and RGDS, corresponding to the gamma and alpha platelet receptor recognition domains of fibrinogen. As is shown by Figures 2A and 2B, these experiments yield IC_{50} values of 30 μM and 10 μM, respectively. IC_{50} values are the concentration of peptide at which there is 50% inhibition of fibrinogen binding.

The results of the same experiment can also be seen as the first two lines under the column entitled ^{125}I-Fibrinogen binding, IC_{50}, on Table III. In addition, Table III also illustrates the

IC_{50} for aggregation of ADP-treated platelet-rich plasma by the same peptides. These values are 300 μM and 50 μM, respectively.

TABLE III

Antiaggregatory Potency of Synthetic Peptides Containing Sequences of Gamma and Alpha Chain of Human Fibrinogen.

Peptide	^{125}I-Fibrinogen Binding IC_{50}	Aggregation in ADP-Treated Platelet-Rich Plasma IC_{50}
YHHLGGAKQAGDV	30	300
RGDS	10	50
YRGDSQHLGGAKQAGDV	30	120
RGDV	6	50
HHLGGAKQAGDSRGDV	10	100
HHLGGAKQAGDVGRGDV	28	150
CYHHLGGAKQRGDV	5	150
RGDF	2	40
KQRGDF	13	125
AKQRGDF	9	75
HHLGGAKQRGDF	6	100

A variety of hybrids having at least a portion of the platelet receptor recognition domain of the gamma chain or analogue thereof and the RGD portion of the platelet receptor recognition domain from the alpha chain were prepared using standard

solid phas methods. In s me f the hybrids, the terminal ph nylalanine (F) or serine (S) of the alpha chain domain was replaced with a valine (V) because of the known effectiveness of valine at the terminal end of the γ400-411 domain.

Figures 3A-G showed testing with a variety of the hybrid peptides of the invention. In Figure 3A, the peptide shown has the analogue of the domain from the alpha chain at the amino rather than carboxyl terminal end. In comparison to the other results, based particularly on inhibition of ^{125}I-Fibrinogen binding, this type of hybrid peptide was deemed to be as effective as native peptide γ400-411.

The experiments shown in Figure 3B-G all relate to tests run with hybrid peptides having a fragment or analogue of the gamma chain binding site in the amino region of the peptide and the alpha chain portion in the carboxyl area. The results indicate that replacing a portion of the gamma chain region with the alpha chain region with the alpha chain region promoted inhibition of ^{125}I-Fibrinogen binding. However, this effect diminishes under only two or three residues form the gamma chain remain.

Turning again to Table III, this diminution effect is shown not just for ^{125}I-Fibrinogen binding but also for platelet aggregation with ADP-treated platelet-rich plasma. However, Table III shows one of the surprising results of use hybrids having both alpha and gamma chain components. Hybrid

peptides having a large portion of the gamma chain component, e.g., HHLGGAKQAGDSRGDV or HHLGGAKQAGDVGRGDV, yield improvement (decrease) in the ratio of the amount necessary to inhibit ^{125}I-Fibrinogen binding to the amount necessary to inhibit platelet aggregation. In fact, the aggregation-preventing amount is as low, or lower, then just the alpha tetrapeptide itself, a surprising finding. This is even more important since a problem with using just an alpha fragment is that the tetrapeptide fragment exerts a significant effect on the integrity of the vascular endothelium in regard to its interaction with the extra cellular matrix. Chen et al., J. Cell Biol., 105, p. 1885 (1987). In vivo, this effect may result in endothelial desquamation and/or impaired endothelialization of the denuded vascular surfaces. However, by making a fusion product of the dodecapeptide (γ400-411) and the alpha chain fragments, the resulting hybrid may lose its reactivity towards vascular endothelium so it may be possible to produce hybrids having enhanced inhibitory potency while retaining the significant features of the gamma chain domain, e.g., fibrinogen and platelet specificity and protection of vascular endothelium.

As is evident from the data presented in Table III, the potency of the hybrid peptides, even when including a cystine near the terminal end, is greater than the gamma chain peptide alone. Since cystine can be used for form disulfide bridges to proteins, or can be dimerized to form multimers, it appears that a synthetic fibrinogen could be formed

using the hybrids of the invention. For example, see United States Pat nt No. 4,703,039. In like manner, the close physical structure connection of binding domains between fibrinogen and von Willebrand factor, see United States Patents Nos. 4,661,471 and 4,666,884, leads to the possibility that the same procedure could be used for making molecules which inhibit von Willebrand factor, fibrin, or other adhesive protein platelet interaction, or, in fact, allow for the formation of synthetic adhesive proteins. In fact, it may be possible to mix domains, e.g., place domains from different peptides on the same hybrid to achieve new and distinct effects.

The foregoing examples are purely illustrative and are not meant to limit the scope of the present invention. Those skilled in the art may determine other methods or peptides within the scope of the invention. The invention is defined by the following claims.

What is claimed is:

CLAIMS:

1. A hybrid linear peptide of the form X_1-X_2 where X_1 comprises an analogue of a first interaction site on an adhesive protein for a first receptor domain for said protein, and X_2 comprises an analogue of a second interaction site on said adhesive protein for a second receptor domain on said protein, wherein said first interaction site and said second interaction site are distinct on the native form of said adhesive protein.

2. The hybrid peptide of claim 1 wherein said first and second receptor domains are located on a platelet.

3. The hybrid peptide of claim 2 wherein said adhesive protein is selected from a group consisting of fibrinogen, fibrin, and von Willebrand factor.

4. The hybrid peptide of claim 3 wherein said adhesive protein is fibrinogen and X_1 and X_2 comprise analogues of the interaction sites for platelets on the gamma chain and the alpha chain of fibrinogen.

5. The hybrid peptide of claim 4 wherein X_1 comprises an analogue or analogue fragment of the gamma chain platelet interaction site and X_2 comprises an analogue or analogue fragment of the alpha chain platelet interaction site of native fibrinogen.

6. The hybrid peptide of claim 5 wherein X_2 comprises a tetrapeptide selected from a group consisting of RGDV, RGDN, RGDF, RGDY, RGDS, RGDM, and RGDC.

7. The hybrid peptide of claim 6 wherein X_1 is a linear peptide of 2-13 residues comprising an active portion of the sequence HHLGGAKQAGDV or substituted analogues thereof.

8. The hybrid peptide of claim 7 wherein said active portion comprises the sequence HHLGGAKQ.

9. A method of inhibiting the interaction of platelets with adhesive proteins comprising the steps of:

forming a linear hybrid peptide which contains analogues of peptide sequences from said adhesive protein which react with at least two different domains of interaction sites on said platelets;

reacting said hybrid peptide with said platelets; and

exposing said platelets to said adhesive protein.

10. The method of claim 9 wherein said hybrid peptide has the form X_1-X_2 where X_1 is an analogue of a first interaction site on said adhesive protein for a first receptor domain for said adhesive

protein, and X_2 is an analogue of a second interaction site in said adhesive protein for a second receptor domain on said adhesive protein.

11. The method of claim 10 wherein said adhesive protein comprises fibrinogen, X_1 comprises an analogue of the interaction site for platelets on the gamma chain of fibrinogen, and X_2 comprises an analogue of the interaction site for platelets on the alpha chain of fibrinogen.

12. The method of claim 11 wherein X_2 comprises a tetrapeptide selected from a group consisting of RGDV, RGDN, RGDF, RGDY, RGDS, RGDM, and RGDC.

13. The method of claim 11 wherein X_1 is a linear peptide of 2-13 residues comprising an active portion of the sequence HHLGGAKQAGDV or substituted analogues thereof.

14. The method of claim 13 wherein said active portion comprises the sequence HHLGGAKQ.

15. The method of claim 9 wherein said adhesive protein comprises von Willebrand factor.

16. A method of promoting aggregation of platelets comprising the steps of:

forming a hybrid aggregation promoting molecule, said hybrid aggregation promoting molecule being a multimer of a linear hybrid analogue of

interaction sites on an adhesiv pr tein which r act with receptor sites n platelets, each of said linear hybrid analogues having the form X_1-X_2 where X_1 is an analogue of at least a portion of a first interaction site on said adhesive protein and X_2 is an analogue of at least a portion of a second interaction site on said adhesive protein; and

incubating said hybrid aggregation promoting molecule with platelets under conditions which promote interaction of said platelets and said hybrid aggregation promoting molecule to promote platelet aggregation.

17. The method of claim 16 wherein said conditions which promote interaction comprise the step of activating said platelets with an agonist.

18. The method of claim 16 wherein said adhesion protein comprises fibrinogen, said first interaction site comprises an analogue of the platelet interaction site on the gamma chain of fibrinogen, and said second interaction site comprises an analogue of the platelet interaction site on the alpha chain of fibrinogen.

19. The method of claim 18 wherein X_2 comprises a tetrapeptide selected from a group consisting of RGDV, RGDN, RGDF, RGDY, RGDS, RGDM, and RGDC.

20. The method of claim 18 wherein X_1 is a linear peptide of 2-13 residues comprising an active

portion of the sequenc HHLGGAKQAGDV or substituted analogues thereof.

21. The method of claim 20 wherein said active portion comprises the sequence HHLGGAKQ.

22. A method of promoting adhesion of platelets to vessel walls comprising the steps of:

forming a hybrid adhesion promoting molecule, said hybrid adhesion promoting molecule being a multimer of a linear hybrid analogue of interaction sites on an adhesive protein which react with receptor sites on platelets, each of said linear hybrid analogues having the form X_1-X_2; and

incubating said hybrid adhesion promoting molecule with platelets under conditions which promote interaction of said platelets and said hybrid adhesion promoting molecule to promote platelet adhesion.

23. The method of claim 22 wherein said conditions which promote interaction comprise the step of activating said platelets with agonists.

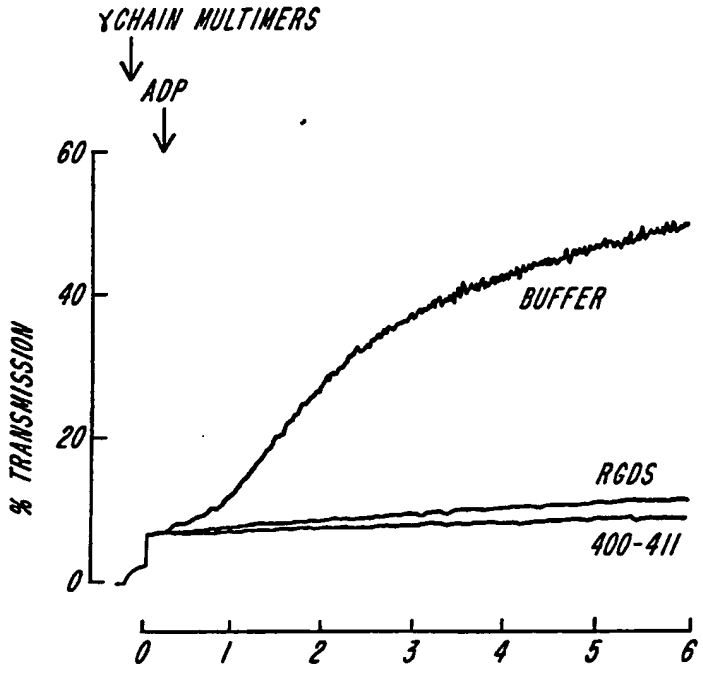
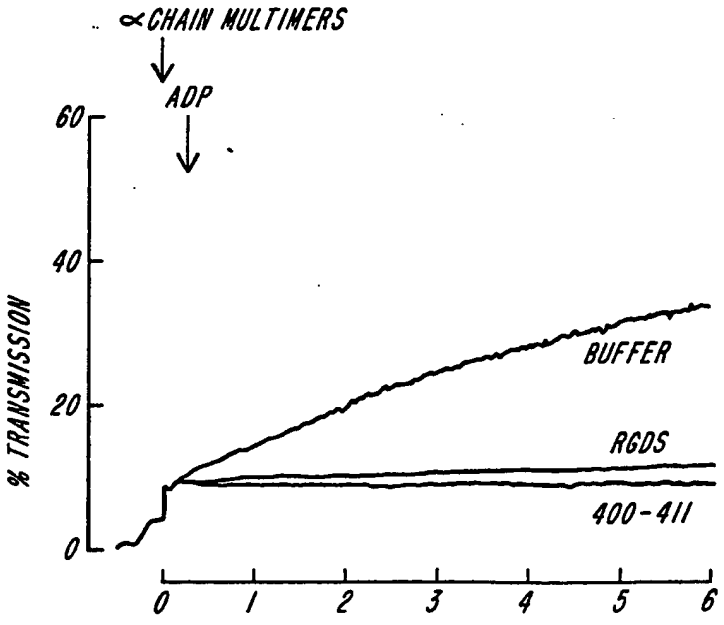

γCHAIN MULTIMERS
ADP
60
40
20
0
% TRANSMISSION
BUFFER
RGDS
400-411
0
1
2
3
4
5
6

FIG. 1A


αCHAIN MULTIMERS
ADP
60
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% TRANSMISSION
BUFFER
RGDS
400-411
0
1
2
3
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5
6

FIG. 1B

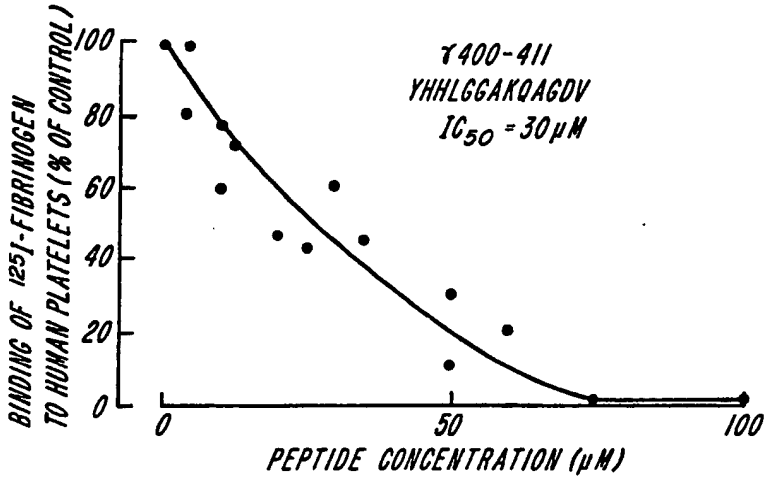
γ400-411
YHHLGGAKQAGDV
IC50 = 30μM
BINDING OF 125I-FIBRINOGEN TO HUMAN PLATELETS (% OF CONTROL)
100
80
60
40
20
0
0
50
100
PEPTIDE CONCENTRATION (μM)

FIG. 2A

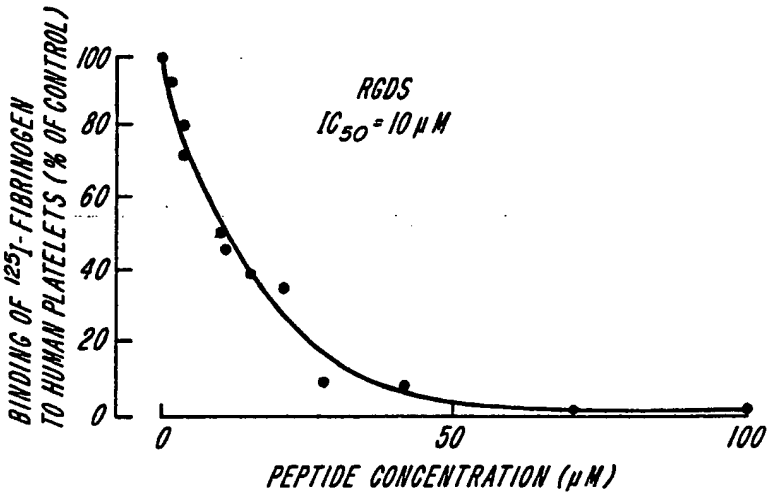
RGDS
IC50 = 10μM
BINDING OF 125I-FIBRINOGEN TO HUMAN PLATELETS (% OF CONTROL)
100
80
60
40
20
0
0
50
100
PEPTIDE CONCENTRATION (μM)

FIG. 2B

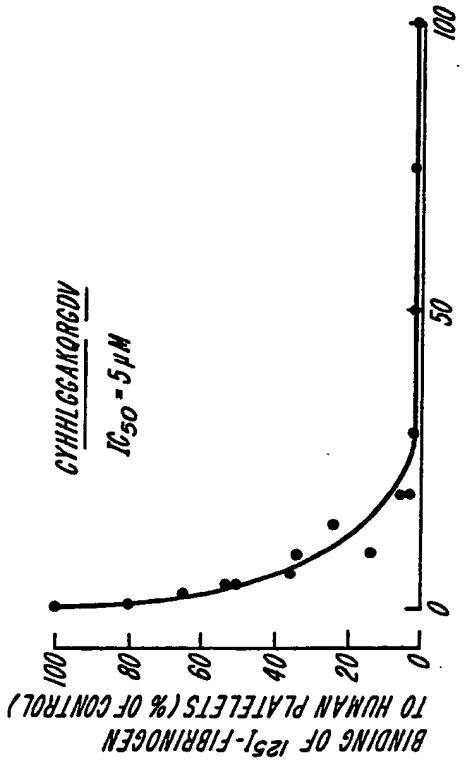

FIG. 3C

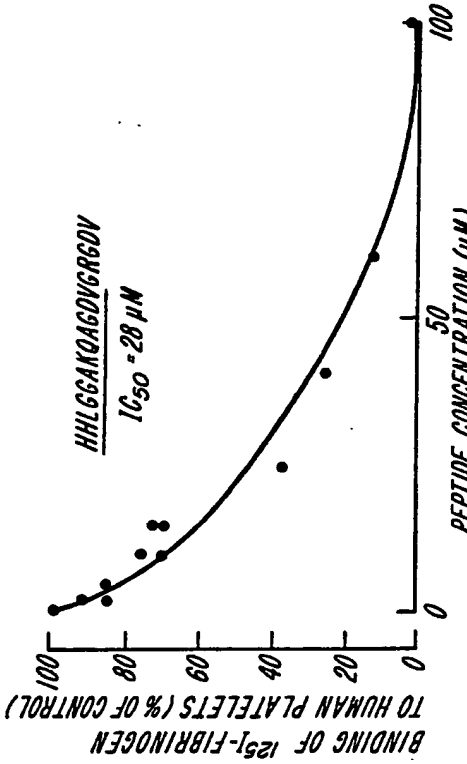

FIG. 3D

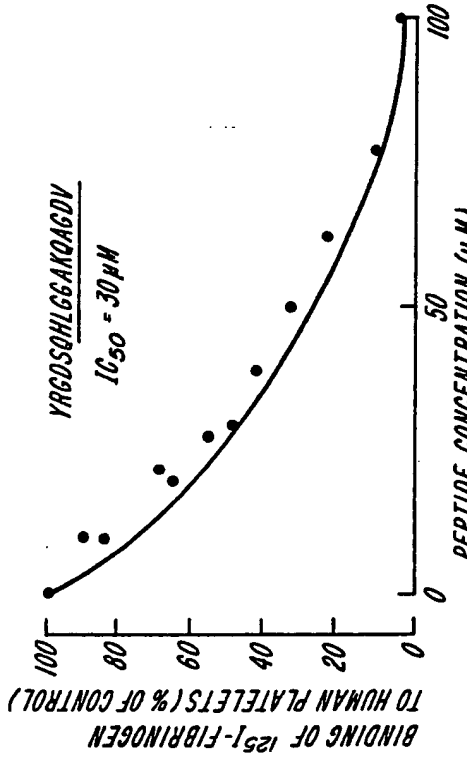

FIG. 3A

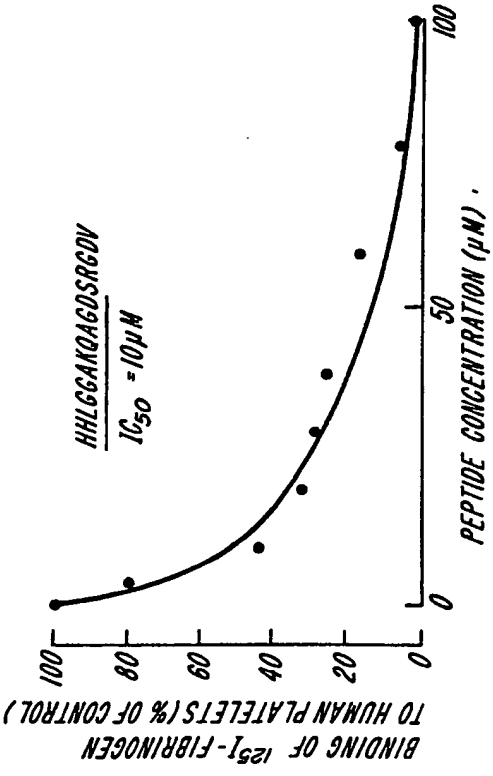

FIG. 3B

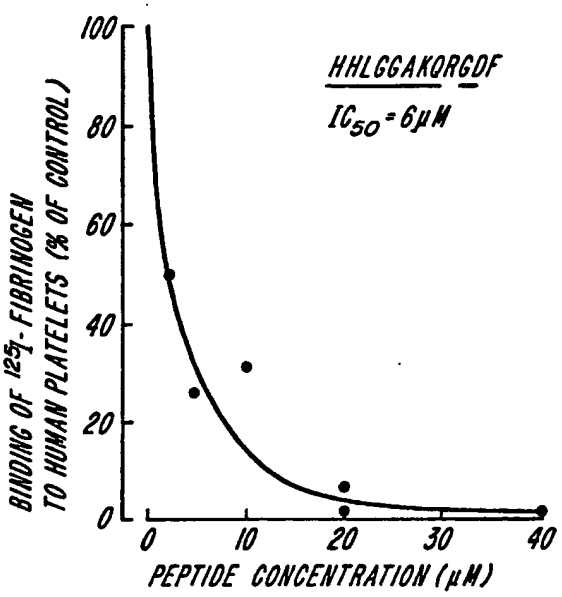

FIG. 3E

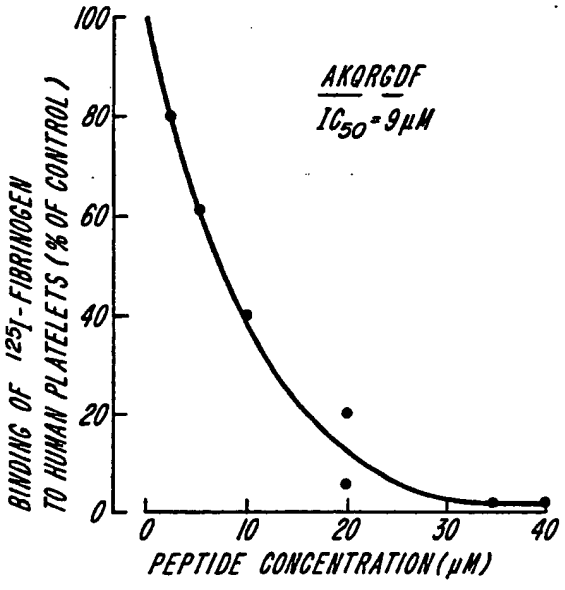

FIG. 3F

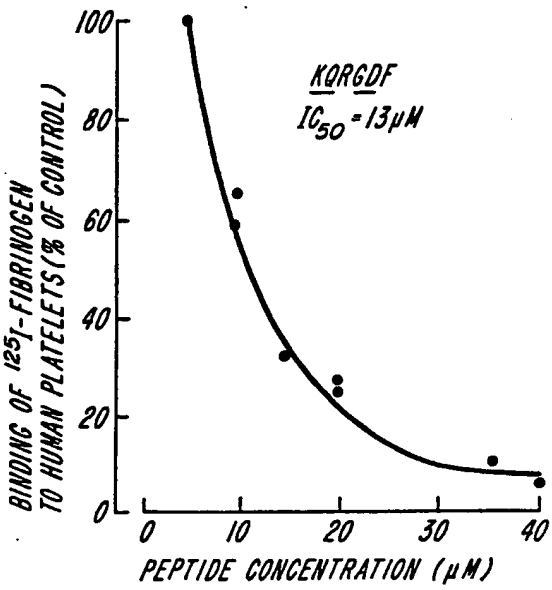

FIG. 3G

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 89/01742

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 37/02; C07K 7/06; C07K 7/08
U.S.: 514/13, 14, 15, 16, 17; 530/326, 327, 328, 329

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	514/13, 14, 15, 16, 17; 530/326, 327, 328, 329

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

~~Databases: Chemical Abstracts Service Online (File CA,~~ 1967-1989); Automated Patent Search System (File USPAT, 1975-1989); Sequence search; See Attachment.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X / Y	Proc. Natl. Acad. Sci. USA. March, 1982 V. 79, pp. 2068-2071, Hawiger. See entire article.	1-8 / 16-23
X / Y	Biochemistry. 1984. V. 23, pp. 1767-1774, Kloczewiak. See page 1769, Table 1	7, 8 / 16-23
X	U.S., A, 4,578,079. Ruoslahti, 25 April 86 See Figure 3 and claim 23.	1-8
X / Y	U.S., A, 4,683,291. Zimmerman, 28 May 87 See columns 6, 7, and 8.	1-15 / 16-23

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19 July 1989	18 AUG 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Nina Ossanna

Form PCT/ISA/210 (second sheet) (Rev. 11-87)

Attachment to Form PCT/ISA/210, Part II.

II. Fields searched search terms:

platelet receptor, hybrid protein, fibrinogen, hybrid peptide, GPIIB/IIIA, recognition domain